Porous Ca-Si-based nanospheres: a potential intra-canal disinfectant-carrier for infected canal treatment

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ABSTRACT

The aim of this study is to develop a new intra-canal disinfectant-carrier for infected canal treatment. To achieve this purpose, a new porous Ca-Si (CS)-based nanosphere was synthesized and characterized. Results showed that the nanospheres can infiltrate into dentinal tubules and released the ampicillin over one week time in a sustained manner. The release of ampicillin from spheres has significantly antibacterial property. Extensive and well-organized *in vitro* mineralization and crystallization of apatite were induced on the surface of dentin slices covered by CS nanospheres. All these features indicate that the porous CS nanospheres may be developed into a new intra-canal disinfectant-carrier for infected canal treatment.

Key words: *disinfectant; root canal treatment; nanosphere; controlled release; mineralization*

1. Introduction

Thorough cleaning of root canal system prior to canal filling is a key pre-requisite for the long-term success of endodontic treatment [1, 2]. This cleaning procedure aims at the complete removal of

dentin debris and disinfection of whole canal system. Remaining bacteria from infected canals, such as *Enterococcus faecalis*, could hide in the fine canal branches, ramifications and dentin tubules, resulting in the post-treatment chronic apical disease or even the treatment failure [2, 3]. This is the exact reason why the intra-canal disinfectant medication is required for the treatment of infected root canals during the multi-visit treatment period. The ideal intra-canal disinfectant should be of the following properties: bactericidal, chemically basic, apical cell-friendly and tissue regeneration-favourite.[4] To solve these problems, an important way is to develop new nano-sized particles with a drug-delivery ability to enter the dentin tubules and other irregular narrow areas. Previous studies have shown that Ca-Si (CS)-based ceramics and cements can be used as bone defect filling materials due to their excellent bioactivity and biocompatibility [5-7] and the Ca and Si ions from materials could stimulate the proliferation and differentiation of osteoblast-like cells [8]. Therefore, the main purpose of this study is to prepare a porous CS nanosphere system and investigate its ability in entering dentin tubules, anti-biotics delivery, *in vitro* antibacterial and mineralization as a potential intra-canal disinfectant-carrier.

2. Experimental

2.1. Fabrication and characterization of porous CS nanospheres

Porous CS nanospheres were synthesized by a hydrothermal method. Briefly, 1.0 g of poly(vincylpyrrolidon) (PVP, K30, Sigma) and 0.46 sodium hydrate were dissolved in 120mL ddH_2O with stirring for 10 min. Then, 1.40g of cetyltrimethylammonium bromide (CTAB) was added for 1h with stirring. 1.1 g of $Ca(NO_3)_2 \cdot 4H_2O$, 5.22 g of tetraethyl orthosilicate and 0.56g of triethyl phosphate (Sigma) were added with vigorous stirring. The mixture was stirred for 24 h, then sealed in Teflon-lined autoclaves at 80°C for 48 h. The products were collected by filtration and wash by ddH_2O and ethanol for 3 times. Then the collected powders were dried at 100 °C overnight and calcined at 550 °C for 5h to remove remained PVP. The obtained porous CS

microspheres were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy dispersive spectrometer (EDS).

2.2. Antibiotics loading and release

25mg of CS nanospheres were soaked in 5mg/mL of ampicillin-phosphate buffer saline (PBS) solution at 4°C overnight. Then the spheres were centrifuged at 10,000 rpm for 10 min and the supernatant were completely removed. The loading amount of ampicillin was determined by UV analysis (at wavelength 230nm) through calculating the difference of ampicillin-PBS concentration before and after loading. For ampicillin releasing test, the collected ampicillin-loaded CS nanospheres were soaked into 4mL fresh PBS at 37°C for different period of time. At each time point, 2mL of PBS solution was taken out to test the released ampicillin and 2mL fresh PBS was added back. The accumulative release percentage of ampicillin from CS spheres was calculated.

2.3. In vitro anti-bacteria test

25mg of CS nanospheres were soaked in 5mg/mL of ampicillin-PBS solution at 4°C overnight to load ampicillin. Then, ampicillin-loaded CS nanospheres were mixed with 5mL *E.coli* (DH5α)-LB culture media ($3.5\text{-}4.0\times10^4$ bacteria/mL) and maintained at 4°C overnight. Then 10μL of mixture was plated into a 10mm culture dish and incubated at 37°C for 12 h. The bacteria without particles or mixed with naked CS nanospheres were used as controls. The test was carried out in triplicate for each group and the *E.coli* colonies on each dish were counted for group comparisons.

2.4. In vitro dentin tubule infiltration test

1mm-thick dentin slices were cut from sheep molars and washed in 10% EDTA solution to remove the dentin debris and smear layer. Then the dentin slices were soaked into the 10 mg/mL CS nanospheres-PBS suspension. 40000Hz ultrasound was applied to the suspension for 5 min. Then the dentin slices were dried at 60 °C for 12 h. The surface morphology of CS nanospheres-loaded dentin slices were observed by SEM. To further confirm CS nanospheres were filled into dentinal tubules, CS nanospheres-loaded dentin slices were frozen at -80°C and broken to expose the axial

cross-section of dentinal tubules. SEM was used to observe the infiltration of CS nanospheres inside the dentinal tubules.

2.5. In vitro mineralization

SBF containing ion concentrations similar to those in human blood plasma was prepared according to the method described by Kokubo [9]. CS nanospheres-loaded dentin slices (10×10 mm) were soaked in 50mL of SBF at 37°C for seven days. Apatite mineralization on the surface of CS nanospheres-loaded dentin was characterized by SEM and EDS. The pH value of SBF over time was tested using a pH meter.

3. Results

3.1. Characterization of porous CS nanospheres

SEM images showed the spherical morphology of the prepared CS nanospheres with a size of 80-150 nm (Fig. 1A). These nanospheres had internal porous structure revealed by TEM images (Fig. 1B). EDS test confirmed the calcium and silicate elements in the chemical component of the particles (Fig. 1C). The Ca/Si ratio in the particles was 0.26±0.02. The weak Au peak (Fig. 1C) is due to gold coating when preparing SEM samples.

3.2. Ampicillin release and anti-bacteria effect

Porous CS nanospheres were found to have a sustained release of ampicillin over a week timeframe (Fig. 2A). In anti-bacteria test, the ampicilin-loaded CS nanospheres showed the highest anti-bacteria effects, while the CS nanosphere itself also showed anti-bacteria effects when compared with blank controls without nanospheres ($p<0.05$, Fig. 2B).

3.3. Dentinal tubules infiltration

SEM images with different magnifications revealed that the particles could be successfully introduced into the dentinal tubules by ultrasound activation (Fig. 3). These particles could not only block the openings of tubules (Fig. 3B and C) but also infiltrate into the internal area of tubules (Fig. 3E and F).

3.4. In vitro mineralization

In vitro mineralization test showed that massive well-organized apatite crystals grew on the CS nanospheres-loaded dentin slices (Fig. 4A and B). The EDS test on these crystals further confirmed they were newly-formed apatite containing Ca, P, Na, Mg and Cl elements (Fig. 4C). The ratio of Ca/P for the mineralized apatite crystals is 1.60, which is similar to that of stoichiometric hydroxylapatite crystals (1.67). The pH value change in SBF induced by the particles over a week time was found slightly basic, especially in the first 24 h (Fig. 4D).

4. Discussion

In this study, a new type of spherical nano-sized particles showed its excellent infiltration ability into dentinal tubules under ultrasound activation, which provides the pre-requisite for its following intra-tubular bactericidal effects. One of the most important features of the new nano-sized particles in this study is its internal porous structure. This structure showed massive nano-pores inside each particle. These nanopores render the particle superior ability to carry chemicals, antibiotics or growth factors, and release them over time in a controlled manner. In this study, the ampicillin was successfully loaded onto particles and gradually released in almost a week timeframe during which a relatively high and constant released amount of amoxicillin was observed. Previous studies had shown that silicate-based biomaterials can easily form Si-OH groups on the surface of materials. Si-OH groups and nanopores of biomaterials will help bind with low-molecule drugs and proteins [10-13]. In this study, it is reasonable that the nanopore structure in CS microspheres and the OH^- groups on their surface helped bind ampicillin and benefitted the sustained release. This ability is of great significance for the inter-visit canal disinfection as during this period, the bactericidal effects can be well-maintained.

In terms of chemical components of the particle, Ca^{2+} and SiO_4^{4-} have been found essential and favourite for bio-mineralization and hard tissue regeneration [14, 15]. In this study, in vitro mineralization test showed this new CS nanospheres induced extensive mineralization and

crystallization. The formation of apatite mineralization may help the occlusion of dentinal tubules, preventing further bacteria infiltration and survival [16].

5. Conclusions

A new porous CS nanosphere was introduced which showed excellent abilities in carrying and releasing antibiotics in a controlled manner. These particles are chemically basic and can infiltrate into dentinal tubules by ultrasound activation. Extensive in vitro mineralization can also be induced by this CS nanosphere. All these features may indicate that porous CS nanospheres could be developed into a new intra-canal disinfectant-carrier for infected canal treatment.

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